# EXHIBIT Z

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(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Ravinder S. DHALLAN

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Examiner: E. Whisenant

For: METHODS FOR DETECTION OF GENETIC DISORDERS

**AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION**

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

## INTRODUCTORY COMMENTS

This is in response to the non-final Office Action dated January 30, 2007 (Part of Paper No./Mail Date 20070105), for which a response was due on April 30, 2007. Filed herewith is a Petition and fee for a one month extension of time, thereby extending the deadline for response to May 30, 2007. Accordingly, this response is timely filed. Reconsideration and allowance of the pending claims, as amended, in light of the remarks presented herein are respectfully requested.

**Amendments to the Claims** are reflected in the listing of claims which begins on page 2 of this paper.

**Remarks/Arguments** begin on page 24 of this paper.

## AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings of claims in the application:

Claim 1 (currently amended): A method for detecting the presence or absence of a fetal chromosomal abnormality, said method comprising: quantitating a ratio of the relative amount of ~~the~~ alleles at a heterozygous locus of interest in a mixture of template DNA, wherein said mixture comprises maternal DNA and fetal DNA, and wherein said mixture of maternal DNA and fetal DNA has been obtained from a sample from a pregnant female, and further wherein said heterozygous locus of interest has been identified by determining the sequence of alleles at [[a]] the locus of interest ~~from template DNA obtained from a sample from a pregnant female, wherein said relative amount is expressed as a ratio~~, and wherein said ratio indicates the presence or absence of a fetal chromosomal abnormality~~, and wherein said template DNA comprises a mixture of maternal DNA and fetal DNA~~.

Claim 2 (original): The method of claim 1, wherein said template DNA is obtained from a source selected from the group consisting of human, non-human, mammal, reptile, cattle, cat, dog, goat, swine, pig, monkey, ape, gorilla, bull, cow, bear, horse, sheep, poultry, mouse, rat, fish, dolphin, whale, and shark.

Claim 3 (original): The method of claim 2, wherein the template DNA is obtained from a human source.

Claim 4 (previously presented): The method of claim 1, wherein the template DNA is obtained from a sample selected from the group consisting of: a blood, serum, plasma, saliva, urine, tear, vaginal secretion, lymph fluid, cerebrospinal fluid, mucosa secretion, peritoneal fluid, ascitic fluid, fecal matter, and body exudates.

Claim 5 (original): The method of claim 1, wherein alleles of multiple loci of interest are sequenced and their relative amounts quantitated and expressed as a ratio.

Claim 6 (original): The method of claim 5, wherein said multiple loci of interest are on multiple chromosomes.

Claim 7 (cancelled)

Claim 8 (currently amended): The method of claim 3, wherein template DNA from said human pregnant female is obtained from a sample selected from the group consisting of: blood, serum, plasma, saliva, urine, tear, vaginal secretion, lymph fluid, cerebrospinal fluid, mucosa secretion, peritoneal fluid, ascitic fluid, fecal matter, and body exudate.

Claim 9 (original): The method of claim 4, wherein said sample is mixed with an agent that inhibits cell lysis to inhibit the lysis of cells, if cells are present, wherein the agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Claim 10 (previously presented): The method of claim 9 wherein said agent is a cell lysis inhibitor.

Claim 11 (original): The method of claim 10, wherein said cell lysis inhibitor is selected from the group consisting of glutaraldehyde, derivatives of glutaraldehyde, formaldehyde, formalin, and derivatives of formaldehyde.

Claim 12 (original): The method of claim 9, wherein said sample is blood.

Claim 13 (cancelled)

Claim 14 (previously presented): The method of claim 12, wherein said blood is obtained from a human pregnant female when the fetus is at a gestational age selected from the group consisting of: 0-4, 4-8, 8-12, 12-16, 16-20, 20-24, 24-28, 28-32, 32-36, 36-40, 40-44, 44-48, 48-52, and more than 52 weeks.

Claim 15 (previously presented): The method of claim 12, wherein said template DNA is obtained from plasma from said blood.

Claim 16 (previously presented): The method of claim 12, wherein said template DNA is obtained from serum from said blood.

Claim 17 (cancelled)

Claim 18 (previously presented): The method of claim 15 or 16, wherein prior to determining the sequence of alleles of a locus of interest from template DNA, maternal DNA is sequenced to identify a homozygous locus of interest, and further wherein said homozygous locus of interest is the locus of interest analyzed in the template DNA.

Claim 19 (previously presented): The method of claim 15 or 16, wherein prior to determining the sequence of alleles of a locus of interest from template DNA, maternal DNA is sequenced to identify a heterozygous locus of interest, and further wherein said heterozygous locus of interest is the locus of interest analyzed in the template DNA.

Claim 20 (original): The method of claim 1, wherein determining the sequence of the alleles comprises:

(a) amplifying alleles of a locus of interest on a template DNA using a first and a second primer, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;

(b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;

(c) incorporating a nucleotide into the digested DNA of (b) by using the 5' overhang containing the locus of interest as a template; and

(d) determining the sequence of the alleles of the locus of interest by determining the sequence of the DNA of (c).

Claim 21 (currently amended): The method of claim 1, wherein determining the sequence of alleles comprises:

(a) amplifying alleles of a locus of interest on a template DNA using a first and second primers, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;

(b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;

(c) incorporating nucleotides into the digested DNA of (b), wherein;

(i) a nucleotide that terminates elongation, and is complementary to an allele of the locus of interest ~~of an allele,~~ is incorporated into the 5' overhang of said allele, and

(ii) a nucleotide complementary to a different allele of the locus of interest ~~of a different allele~~ is incorporated into the 5' overhang of said different allele, and said terminating nucleotide, which is complementary to a nucleotide in the 5' overhang of said different allele, is incorporated into the 5' overhang of said different allele.

(d) determining the sequence of the alleles of a locus of interest by determining the sequence of the DNA of (c).

Claim 22 (original): The method of claim 20 or 21, wherein the incorporation of a nucleotide in (c) is by a DNA polymerase selected from the group consisting of E. coli DNA

polymerase, Klenow fragment of E. coli DNA polymerase I, T7 DNA polymerase, T4 DNA polymerase, Taq polymerase, Pfu DNA polymerase, Vent DNA polymerase and sequenase.

Claim 23 (original): The method of claim 20, wherein the incorporation of a nucleotide in (c) comprises incorporation of a labeled nucleotide.

Claim 24 (original): The method of claim 20, wherein the incorporation of a nucleotide in (c) comprises incorporation of a dideoxynucleotide.

Claim 25 (original): The method of claim 20, wherein the incorporation of a nucleotide in (c) further-comprises incorporation of a deoxynucleotide and a dideoxynucleotide.

Claim 26 (currently amended): The method of claim [[1]] 20, wherein the incorporation of a nucleotide in (c) further comprises using a mixture of labeled and unlabeled nucleotides.

Claim 27 (original): The method of claim 23, wherein the labeled nucleotide is labeled with a molecule selected from the group consisting of radioactive molecule, fluorescent molecule, antibody, antibody fragment, hapten, carbohydrate, biotin, derivative of biotin, phosphorescent moiety, luminescent moiety, electrochemiluminescent moiety, chromatic moiety, and moiety having a detectable electron spin resonance, electrical capacitance, dielectric constant and electrical conductivity.

Claim 28 (original): The method of claim 27, wherein the labeled nucleotide is labeled with a fluorescent molecule.

Claim 29 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(i) comprises incorporation of a labeled nucleotide.

Claim 30 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(i) comprises incorporation of a dideoxynucleotide.

Claim 31 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(i) further comprises incorporation of a deoxynucleotide and a dideoxynucleotide.

Claim 32 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(i) further comprises using a mixture of labeled and unlabeled nucleotides.

Claim 33 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(ii) comprises incorporation of a labeled nucleotide.

Claim 34 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(ii) comprises incorporation of a deoxynucleotide.

Claim 35 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(ii) further comprises incorporation of a deoxynucleotide and a dideoxynucleotide.

Claim 36 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(ii) further comprises using a mixture of labeled and unlabeled nucleotides.

Claim 37 (original): The method of claim 29, wherein the labeled nucleotide is a dideoxynucleotide.

Claim 38 (original): The method of claim 29, wherein the labeled nucleotide is labeled with a molecule selected from the group consisting of radioactive molecule, fluorescent molecule, antibody, antibody fragment, hapten, carbohydrate, biotin, derivative of biotin, phosphorescent moiety, luminescent moiety, electrochemiluminescent moiety, chromatic moiety, and moiety having

a detectable electron spin resonance, electrical capacitance, dielectric constant and electrical conductivity.

Claim 39 (original): The method of claim 38, wherein the labeled nucleotide is labeled with a fluorescent molecule.

Claim 40 (original): The method of claim 39, wherein the incorporation of a nucleotide in (c)(i) further comprises incorporation of an unlabeled nucleotide.

Claim 41 (original): The method of claim 20 or 21, wherein the determination of the sequence of the locus of interest in (d) comprises detecting a nucleotide.

Claim 42 (original): The method of claim 20 or 21, wherein said first and second primers contain a portion of a restriction enzyme recognition site that contains a variable nucleotide, wherein the full restriction enzyme recognition site is generated after amplification.

Claim 43 (original): The method of claim 20 or 21, wherein the restriction enzyme recognition site is for a restriction enzyme selected from the group consisting of BsaJ I, Bssk I, Dde I, EcoN I, Fnu4H I, Hinf I, and ScrF I.

Claim 44 (original): The method of claim 20 or 21, wherein the restriction enzyme cuts DNA at a distance from the recognition site.

Claim 45 (original): The method of claim 44, wherein the recognition site is for a Type IIS restriction enzyme.

Claim 46 (original): The method of claim 45, wherein the Type IIS restriction enzyme is selected from the group consisting of: Alw I, Alw26 I, Bbs I, Bbv I, BceA I, Bmr I, Bsa I, Bst71 I, BsmA I, BsmB I, BsmF I, BspM I, Ear I, Fau I, Fok I, Hga I, Ple I, Sap I, SSfaN I, and Sthi32 I.

Claim 47 (original): The method of claim 20 or 21, wherein said method of amplification is selected from the group consisting of: polymerase chain reaction, self-sustained sequence reaction, ligase chain reaction, rapid amplification of cDNA ends, polymerase chain reaction and ligase chain reaction, Q-beta phage amplification, strand displacement amplification, and splice overlap extension polymerase chain reaction.

Claim 48 (original): The method of claim 47, wherein said method of amplification is PCR.

Claim 49 (original): The method of claim 48, wherein an annealing temperature for cycle 1 of PCR is about the melting temperature of the portion of the 3' region of the second primer that anneals to the template DNA.

Claim 50 (original): The method of claim 49, wherein an annealing temperature for cycle 2 of PCR is about the melting temperature of the portion of the 3' region of the first primer that anneals to the template DNA.

Claim 51 (original): The method of claim 50, wherein an annealing temperature for the remaining cycles of PCR is at about the melting temperature of the entire second primer.

Claim 52 (previously presented): The method of claim 1, wherein determining the sequence comprises a method selected from the group consisting of: allele specific PCR, mass spectrometry, hybridization, primer extension, fluorescence resonance energy transfer (FRET), sequencing, Sanger dideoxy sequencing, DNA microarray, southern blot, slot blot, dot blot, and MALDI-TOF mass spectrometry.

Claim 53 (original): The method of claim 1, wherein said ratio for alleles at heterozygous loci of interest on a chromosome are summed and compared to the ratio for alleles at

heterozygous loci of interest on a different chromosome, wherein a difference in ratios indicates the presence of a chromosomal abnormality.

Claim 54 (original): The method of claim 53, wherein the chromosomes that are compared are human chromosomes selected from the group consisting of: chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X, and Y.

Claim 55 (original): The method of claim 53, wherein the ratio for the alleles at heterozygous loci of interest of chromosomes 13, 18, and 21 are compared.

Claim 56 (original): The method of claim 1, wherein said locus of interest is a single nucleotide polymorphism.

Claim 57 (original): The method of claim 1, wherein said locus of interest is a mutation.

Claim 58 (currently amended): A method comprising determining the sequence of a locus of interest on free fetal DNA isolated from a sample obtained from a pregnant female, wherein said sample comprises ~~comprising~~ free fetal DNA and ~~, wherein~~ an agent that inhibits [[cell]] lysis of cells ~~has been added to said sample to inhibit lysis of cells~~, if cells are present, wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Claim 59 (previously presented): The method of claim 58, wherein said sample is selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 60 (original): The method of claim 59, wherein said sample is blood.

Claim 61 (original): The method of claim 58, wherein said sample comprises free maternal template DNA and free fetal template DNA.

Claim 62 (original): The method of claim 58, wherein said agent is a cell lysis inhibitor.

Claim 63 (original): The method of claim 62, wherein said cell lysis inhibitor is selected from the group consisting of: glutaraldehyde, derivatives of glutaraldehyde, formaldehyde, derivatives of formaldehyde, and formalin.

Claim 64 (currently amended): The method of claim 58, wherein prior to determining the sequence, template DNA [[was]] is isolated.

Claim 65 (original): The method of claim 60, wherein said template DNA is obtained from plasma of said blood.

Claim 66 (original): The method of claim 60, wherein said template DNA is obtained from serum of said blood.

Claim 67 (currently amended): The method of claim 58, wherein prior to determining the sequence of the locus of interest on fetal DNA, the sequence of the locus of interest on maternal template DNA [[was]] is determined.

Claim 68 (currently amended): The method of claim 58, wherein prior to determining the sequence of the locus of interest on fetal DNA, the sequence of the locus of interest on paternal template DNA [[was]] is determined.

Claim 69 (original): The method of claim 58, wherein said locus of interest is a single nucleotide polymorphism.

Claim 70 (original): The method of claim 58, wherein said locus of interest is a mutation.

Claim 71 (original): The method of claim 58, wherein the sequence of multiple loci of interest is determined.

Claim 72 (original): The method of claim 71, wherein the multiple loci of interest are on multiple chromosomes.

Claim 73 (original): The method of claim 58, wherein determining the sequence comprises:

(a) amplifying a locus of interest on a template DNA using a first and second primers, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;

(b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;

(c) incorporating a nucleotide into the digested DNA of (b) by using the 5' overhang containing the locus of interest as a template; and

(d) determining the sequence of the locus of interest by determining the sequence of the DNA of (c).

Claim 74 (original): The method of claim 58, wherein determining the sequence comprises:

(a) amplifying alleles of a locus of interest on a template DNA using a first and second primers, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;

(b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;

(c) incorporating nucleotides into the digested DNA of (b), wherein;

(i) a nucleotide that terminates elongation, and is complementary to the locus of interest of an allele, is incorporated into the 5' overhang of said allele, and

(ii) a nucleotide complementary to the locus of interest of a different allele is incorporated into the 5' overhang of said different allele, and said terminating nucleotide, which is complementary to a nucleotide in the 5' overhang of said different allele, is incorporated into the 5' overhang of said different allele.

(d) determining the sequence of the alleles of a locus of interest by determining the sequence of the DNA of (c).

Claim 75 (original): The method of claim 73 or 74, wherein the restriction enzyme cuts DNA at a distance from the recognition site.

Claim 76 (original): The method of claim 75, wherein the recognition site is for a Type IIS restriction enzyme.

Claim 77 (original): The method of claim 76, wherein the Type IIS restriction enzyme is selected from the group consisting of: Alw I, Alw26 I, Bbs I, Bbv I, BceA I, Bmr I, Bsa I, Bst71 I, BsmA I, BsmB I, BsmF I, BspM I, Ear I, Fau I, Fok I, Hga I, Ple I, Sap I, SSfaN I, and Sthi32 I.

Claim 78 (original): The method of claim 73 or 74, wherein said method of amplification is selected from the group consisting of: polymerase chain reaction, self-sustained sequence reaction, ligase chain reaction, rapid amplification of cDNA ends, polymerase chain reaction and ligase chain reaction, Q-beta phage amplification, strand displacement amplification, and splice overlap extension polymerase chain reaction.

Claim 79 (original): The method of claim 78, wherein said method of amplification is by PCR.

Claim 80 (currently amended): The method of claim 79, wherein an annealing temperature for cycle 1 of PCR is about the melting temperature of the ~~portion of the 3' region of the~~ second primer 3' region that anneals to the template DNA.

Claim 81 (currently amended): The method of claim 80, wherein an annealing temperature for cycle 2 of PCR is about the melting temperature of ~~the portion of the 3' region of~~ the first primer 3' region that anneals to the template DNA.

Claim 82 (original): The method of claim 81, wherein an annealing temperature for the remaining cycles of PCR is at about the melting temperature of the entire second primer.

Claim 83 (currently amended): The method of claim 58, wherein the sequence of a locus of interest [[was]] is determined using a method selected from the group consisting of: allele specific PCR, mass spectrometry, hybridization, primer extension, fluorescence polarization, fluorescence resonance energy transfer (FRET), fluorescence detection, sequencing, Sanger dideoxy sequencing, DNA microarray, southern blot, slot blot, dot blot, and MALDI-TOF mass spectrometry.

Claims 84-86 (cancelled)

Claim 87 (currently amended): A method for preparing a sample for analysis comprising isolating free fetal nucleic acid from ~~a~~ the sample ~~that contains nucleic acid~~, wherein said sample comprises an agent that inhibits [[cell]] lysis of cells ~~has been added to the sample to inhibit lysis of cells~~, if cells are present, and wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Claim 88 (original): The method of claim 87, wherein said sample is obtained from a source selected from the group consisting of human, non-human, mammal, reptile, cattle, cat, dog, goat, swine, pig, monkey, ape, gorilla, bull, cow, bear, horse, sheep, poultry, mouse, rat, fish, dolphin, whale, and shark.

Claim 89 (original): The method of claim 88, wherein the sample is obtained from a human source.

Claim 90 (previously presented): The method of claim 87, wherein the sample is obtained from a source selected from the group consisting of:-blood, serum, plasma, saliva, urine, tear, vaginal secretion, lymph fluid, cerebrospinal fluid, mucosa secretion, peritoneal fluid, ascitic fluid, fecal matter, and body exudates.

Claim 91 (original): The method of claim 90, wherein said sample is blood.

Claim 92 (original): The method of claim 91, wherein said blood is from a pregnant female.

Claim 93 (original): The method of claim 92, wherein said blood is obtained from a human pregnant female when the fetus is at a gestational age selected from the group consisting of: 0-4, 4-8, 8-12, 12-16, 16-20, 20-24, 24-28, 28-32, 32-36, 36-40, 40-44, 44-48, 48-52, and more than 52 weeks.

Claim 94 (original): The method of claim 93, wherein said sample is obtained from plasma from said blood.

Claim 95 (original): The method of claim 87, wherein said agent is a cell lysis inhibitor.

Claim 96 (original): The method of claim 87, wherein said cell lysis inhibitor is selected from the group consisting of glutaraldehyde, derivatives of glutaraldehyde, formaldehyde, formalin, and derivatives of formaldehyde.

Claim 97 (original): The method of claim 96, wherein said cell lyis inhibitor is formalin.

Claim 98 (original): The method of claim 97, wherein the final concentration of formalin in the sample is selected from the group consisting of: 0.0001-0.03%, 0.03-0.05%, 0.05-0.08%, 0.08-0.1%, 0.1-0.3%, 0.3-0.5%, 0.5-0.7%, 0.7-0.9%, 0.9-1.2%, 1.2-1.5%, 1.5-2%, and 2-3%.

Claim 99 (original): The method of claim 98, wherein the final concentration of formalin in the sample is 0.1%.

Claim 100 (original): The method of claim 87, wherein isolation of nucleic acid comprises a centrifugation step.

Claim 101 (original): The method of claim 100, wherein the centrifugation step is performed with the centrifuge braking power set to zero.

Claim 102 (original): The method of claim 100, wherein the centrifugation step is performed at a speed selected from the group consisting of 0-50 rpm, 50-100 rpm, 100-200 rpm, 200-300 rpm, 300-400 rpm, 400-500 rpm, 500-600 rpm, 600-700 rpm, 700-800 rpm, 800-900 rpm, 900-1000 rpm, 1000-2000 rpm, 2000-3000 rpm, 3000-4000 rpm, 4000-5000 rpm, 5000-6000 rpm, 6000-7000 rpm, 7000-8000 rpm, and greater than 8000 rpm.

Claims 103-131 (cancelled)

Claim 132 (previously presented): The method of claim 1, wherein said sequence is determined by a method comprising:

(1) amplification of the locus of interest;

(2) exonuclease treatment of the products of (1);

(3) single stranded DNA of (2) is annealed to an oligonucleotide to form an annealed template and primer;

(4) incorporation of a nucleotide using the annealed template and primer of (3);

(5) detection of the incorporated nucleotide.

Claim 133 (previously presented): The method of claim 58, wherein said sequence is determined by a method comprising:

(1) amplification of the locus of interest;

(2) exonuclease treatment of the products of (1);

(3) single stranded DNA of (2) is annealed to an oligonucleotide to form an annealed template and primer;

(4) incorporation of a nucleotide using the annealed template and primer of (3);

(5) detection of the incorporated nucleotide.

Claim 134 (original): The method of claim 132 or 133, wherein the amplification method is selected from the group consisting of: polymerase chain reaction, self-sustained sequence reaction, ligase chain reaction, rapid amplification of cDNA ends, polymerase chain reaction and ligase chain reaction, Q-beta phage amplification, strand displacement amplification, and splice overlap extension polymerase chain reaction.

Claim 135 (original): The method of claim 134, wherein said method of amplification is by PCR.

Claim 136 (original): The method of claim 132 or 133, wherein said primer hybridizes adjacent to the locus of interest.

Claim 137 (original): The method of claim 132 or 133, wherein said incorporated nucleotide is a dideoxynucleotide or deoxynucleotide.

Claim 138 (original): The method of claim 132 or 133, wherein said incorporation reaction comprises two terminating nucleotides and two non-terminating nucleotides.

Claim 139 (original): The method of claim 137, wherein said incorporated nucleotide is labeled with a molecule selected from the group consisting of radioactive molecule, fluorescent molecule, antibody, antibody fragment, hapten, carbohydrate, biotin, derivative of biotin, phosphorescent moiety, luminescent moiety, electrochemiluminescent moiety, chromatic moiety, and moiety having a detectable electron spin resonance, electrical capacitance, dielectric constant and electrical conductivity.

Claim 140 (original): The method of claim 138, wherein said terminating nucleotides are labeled with a molecule selected from the group consisting of radioactive molecule, fluorescent molecule, antibody, antibody fragment, hapten, carbohydrate, biotin, derivative of biotin, phosphorescent moiety, luminescent moiety, electrochemiluminescent moiety, chromatic moiety, and moiety having a detectable electron spin resonance, electrical capacitance, dielectric constant and electrical conductivity.

Claim 141 (original): The method of claim 139, wherein the labeled nucleotide is labeled with a fluorescent molecule.

Claim 142 (original): The method of claim 140, wherein the terminating nucleotides are labeled with a fluorescent molecule.

Claim 143 (original): The method of claim 1, wherein said sequence is determined by a method comprising:

(1) amplification of the locus of interest, wherein the amplification reaction comprises a forward primer, a reverse primer, and a probe that anneals to the locus of interest, which is within the region of the amplicon; and

(2) detection of the PCR products, wherein the amount of PCR product is used to determine the presence or absence of a specific genetic sequence.

Claim 144 (original): The method of claim 58, wherein said sequence is determined by a method comprising:

(1) amplification of the locus of interest, wherein the amplification reaction comprises a forward primer, a reverse primer, and a probe that anneals to the locus of interest, which is within the region of the amplicon; and

(2) detection of the PCR products, wherein the amount of PCR product is used to determine the presence or absence of a specific genetic sequence.

Claim 145 (original): The method of claim 143 or 144, wherein the amplification is by PCR.

Claim 146 (original): The method of claim 143 or 144, wherein the probe contains a reporter dye at the 5' end and the 3' end contains a quenching dye.

Claim 147 (cancelled)

Claim 148 (currently amended): The method of claims 132 or 143, wherein an agent that inhibits cell lysis ~~has been~~ is added to the sample to inhibit the lysis of cells, if present, and wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Claim 149 (previously presented): The method of claim 148, wherein said agent is a cell lysis inhibitor.

Claim 150 (previously presented): The method of claim 149, wherein said cell lysis inhibitor is formalin at a percentage selected from the group consisting of: 0.0001-0.03%, 0.03-0.05%, 0.05-0.08%, 0.08-0.1%, 0.1-0.3%, 0.3-0.5%, 0.5-0.7%, 0.7-0.9%, 0.9-1.2%, 1.2-1.5%, 1.5-2%, and 2-3%.

Claim 151 (previously presented): The method of claim 150, wherein the concentration of formalin in the sample is 0.1%.

Claim 152 (currently amended): A method for detecting the presence or absence of a fetal chromosomal abnormality, said method comprising:

(a) determining the sequence of alleles of a locus of interest from template DNA, wherein the template DNA comprises a mixture of fetal DNA and maternal DNA, and wherein the template DNA is from a sample from a pregnant female,

(b) quantitating a ratio of the relative amount of the alleles in the mixture of fetal DNA and maternal DNA at a heterozygous locus of interest in the mixture that was identified ~~from the locus of interest of~~ by step (a), ~~wherein said relative amount is expressed as a ratio,~~ and wherein said ratio indicates the presence or absence of a fetal chromosomal abnormality.

Claims 153-180 (cancelled)

Claim 181 (previously presented): The method of claim 1, wherein the sample is selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 182 (previously presented): The method of claim 181, wherein the sample is blood.

Claim 183 (previously presented): The method of claim 182, wherein the template DNA is obtained from plasma from said blood.

Claim 184 (previously presented): The method of claim 182, wherein the template DNA is obtained from serum from said blood.

Claim 185 (previously presented): The method of claim 8, wherein template DNA from said human pregnant female is obtained from a sample selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 186 (previously presented): The method of claim 185, wherein the sample is blood.

Claim 187 (previously presented): The method of claim 186, wherein the template DNA is obtained from plasma from said blood.

Claim 188 (previously presented): The method of claim 186, wherein the template DNA is obtained from serum from said blood.

Claim 189 (previously presented): The method of claim 11, wherein said cell lysis inhibitor is selected from glutaraldehyde, formaldehyde and formalin.

Claim 190 (previously presented): The method of claim 58, wherein the sample was obtained from a pregnant female.

Claim 191 (previously presented): The method of claim 190, wherein the pregnant female is human.

Claim 192 (previously presented): The method of claim 191, wherein said sample is selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 193 (previously presented): The method of claim 192, wherein said sample is blood.

Claim 194 (previously presented): The method of claim 193, wherein the free fetal DNA is obtained from plasma from said blood.

Claim 195 (previously presented): The method of claim 193, wherein the free fetal DNA is obtained from serum from said blood.

Claim 196 (previously presented): The method of claim 63, wherein said cell lysis inhibitor is selected from glutaraldehyde, formaldehyde and formalin.

Claims 197-200 (cancelled)

Claim 201 (previously presented): The method of claim 96, wherein said cell lysis inhibitor is selected from the group consisting of glutaraldehyde, formaldehyde, and formalin.

Claim 202 (previously presented): The method of claim 152, wherein the sample is selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 203 (previously presented): The method of claim 202, wherein the sample is blood.

Claim 204 (previously presented): The method of claim 203, wherein the template DNA is obtained from serum from a blood sample from said female.

Claim 205 (previously presented): The method of claim 203, wherein the template DNA is obtained from plasma from a blood sample from said female.

Claim 206 (previously presented): The method of claim 133 or 144, wherein said agent is a cell lysis inhibitor.

Claim 207 (previously presented): The method of claim 1 or 152, wherein said mixture comprises at least about 15% fetal DNA.

Claim 208 (previously presented): The method of claim 1 or 152, wherein said mixture comprises a maximum of about 98-99% fetal DNA.

## REMARKS

Claims 1-6, 8-12, 14-16, 18-83, 87-102, 132-146, 148-152, 181-196 and 201-208 are pending in the present application. Claims 25, 31, 35, 40, 42 and 53-55 are objected to and claims 7, 13, 17, 84-86, 103-131, 147, 153-180 and 197-200 have previously been cancelled. By virtue of this response, claims 1, 8, 21, 26, 58, 64, 67-68, 80-81, 83, 87, 148 and 152 are amended. Accordingly, claims 1-6, 8-12, 14-16, 18-83, 87-102, 132-146, 148-152, 181-196 and 201-208 are currently under consideration. Allowance of the pending claims is respectfully requested.

With respect to all amendments, Applicant has not dedicated or abandoned any unclaimed subject matter and, moreover, has not acquiesced to any rejections and/or objections made by the Patent Office. Applicant reserves the right to pursue prosecution of any presently excluded claim embodiments in future continuation and/or divisional applications.

***Interview Summary***

A telephone interview with Examiner Whisenant was held on May 4, 2007. In addition to Examiner Whisenant and Alicia Hager (the undersigned), Gladys Monroy, Ravinder Dhallan and Michael Cronin participated in the interview. Applicant and his representatives would again like to thank Examiner Whisenant for the courtesy of extending a telephonic interview.

The subject of the telephonic interview was the Office Action dated January 30, 2007, and Applicants' proposed response to the Office Action. Proposed amendments to the independent claims were discussed. In addition, the references cited in the Office Action dated January 30, 2007 was discussed in a general manner.

***Claim Amendments***

The amendment to claims 1, 8, 21, 26, 58, 64, 67-68, 80-81, 83, 87, 148 and 152 are fully supported in the original application.

Claim 1 has been amended to more clearly indicate that the alleles, which are quantified and used to calculate a ratio, comprise a mixture of maternal DNA and fetal DNA, and that the mixture is obtained from a sample from a pregnant female. Support for these amendments can be found at least at paragraphs [0155], [0157], and [0163] of the application and in original claims 7 and 17 (now cancelled).

Claim 8 has been amended to provide proper antecedent basis.

Claim 21 has been amended to clarify the claim language. No new matter has been added.

Claim 26 has been amended to correct the dependency of this claim.

Claim 58 has been amended to indicate that the locus of interest on free fetal DNA is isolated from a sample obtained from a pregnant female. Support for this amendment can be found at least at paragraphs [0064] and [0071]. In addition, elements of the claim have been re-phrased to more clearly indicate the claimed invention.

Claim 64, 67, 68, 83 and 148 have been amended to incorporate a minor change in verb tense, which more clearly indicates the claimed invention.

Claims 80-81 have been amended to provide proper antecedent basis. Support for this amendment can be found at least at paragraph [0201] in the application.

Claim 87 has been amended to indicate that the sample is obtained from a pregnant female and analysis is performed on free nucleic acid. Support for this amendment can be found at least at paragraphs [0064] and [0071] in the application.

Claim 152 has been amended to indicate more clearly that the alleles, which are quantified and used to calculate a ratio, comprises a mixture of maternal DNA and fetal DNA, and that the mixture is obtained from a sample from a pregnant female. Support for these amendments

can be found at least at paragraphs [0155], [0157], and [0163] of the application and in original claims 7 and 17 (now cancelled).

***Claim Objections***

Claims 18-19, 58, 64, 67-68, 83, 87 and 148 are objected to for the following minor informalities:

Applicant is advised that should Claim 18 be allowable, Claim 19 would be objected to under 37 CFR 1.75 as being a substantial duplicate thereof.

Claim 58 is objected to because of the phrase "has been added" should read "is added."

Claim 64 is objected to because of the phrase "was isolated" should read "is isolated."

Claims 67-68 are objected to because of the phrase "was determined" should read "is determined."

Claim 83 is objected to because of the phrase "was determined" should read "is determined."

Claim 87 is objected to because of the phrase "has been added" should read "is added."

Claim 148 is objected to because of the phrase "has been added" should read "is added."

*Claim 18-19*: In response, claim 18 recites a method wherein prior to analyzing the mixture of maternal DNA and fetal DNA, maternal DNA is sequenced to identify a homozygous locus of interest, and further wherein the identified locus of interest is further analyzed in the mixture of maternal DNA and fetal DNA. This identified homozygous maternal locus of interest then is analyzed in the mixture of maternal DNA and fetal DNA. If this locus of interest displays a heterozygous genotype in the mixture, a ratio for the alleles is calculated. For instance, if at a particular locus of interest, the maternal genome is G/G, then this locus of interest will be analyzed

in the mixture of maternal DNA and fetal DNA. If the locus of interest displays a heterozygous genotype in the mixture, such as G/A, a ratio will be calculated for this locus of interest. In this scenario, the fetal allele is readily distinguished (maternal genome is G/G, mixture is G/A), thus, the A allele represents the fetal genome.

On the other hand, claim 19 recites a method wherein maternal DNA is sequenced to identify a heterozygous locus of interest, and the heterozygous locus of interest then is analyzed in the mixture of maternal DNA and fetal DNA. In this scenario, the maternal genome will be heterozygous (for example, A/G) and the locus of interest in the mixture will also be heterozygous (A/G). In this case, there is no allele that distinctly represents the fetal genome. Thus, the claimed matter of claims 18 and 19 are quite distinct.

*Claims 58, 64, 67-68, 83, 87, and 148*: Claims 58 has been amended as is discussed above; the amendments render this objection moot. Claims 64, 67-68, 83, 87, and 148 have been amended to incorporate a minor change in verb tense, which more clearly indicates the claimed invention.

*Claims 25, 31, 35, 40, 42, and 53-55*: Claims 25, 31, 35, 40, 42 and 53-55 are objected to as being dependent upon a rejected base claim, but would appear to be allowable if rewritten in independent form, including all of the limitations of the base claim and any intervening claims.

In response, claims 25, 31, 35, 40, 42 and 53-55 all depend from claim 1, which has been amended. In addition, Applicant has traversed the Examiner's rejection of claim 1. Therefore, Applicant respectfully submits that claims 25, 31, 35, 40, 42 and 53-55 are in condition for allowance.

***Claim Rejections Under 35 U.S.C. § 112 – Second Paragraph***

Claims 26, 80-82 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicant regards as the invention.

*Claim 26:* Claim 26 stands rejected as being indefinite because the phrase "the incorporation of a nucleotide in (c)" lacks proper antecedent basis. Claim 26 has been amended to depend from claim 20. Applicant respectfully submits that the proper basis for the phrase "the incorporation of a nucleotide in (c)"can be fond in claim 20. Thus, Applicant respectfully requests that the rejection of claim 26 under 35 USC 112, 2nd paragraph be withdrawn.

*Claim 80-81*: Claims 80-81 stand rejected as being indefinite because the phrase "the portion of the 3' region" lacks proper antecedent basis. As amended, claims 80-81 no longer lack proper antecedent basis. Applicant respectfully requests that the rejection of claims 80-81 under 35 U.S.C. § 112, 2nd paragraph, be withdrawn.

*Claim 82*: Claim 82 stands rejected under 35 U.S.C. § 112 2nd paragraph as being indefinite for failing to particularly point and distinctly claim the subject matter that the Applicant regards as the invention. Claim 82 depends from claim 81, which has been amended and no longer lacks proper antecedent basis. Applicant respectfully requests that the rejection of claim 82 under 35 U.S.C. § 112, 2nd paragraph, be withdrawn.

**Claims Rejections Under 35 U.S.C. § 103**

*Claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208*: The Office has rejected claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 as being unpatentable over Umansky *et al.* [US 2002/0119478 (2002)] in view of Saiki *et al.* (NEJM 319(9): 537-541 (1988)). Applicant respectfully traverses this rejection.

Claim 1, as amended, is directed to a method for detecting the presence or absence of a fetal chromosomal abnormality, comprising: quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA at a heterozygous locus of interest in the mixture, wherein the mixture of maternal DNA and fetal DNA has been obtained from a sample from a pregnant female, wherein said heterozygous locus of interest has been identified by determining the sequence of alleles at the locus of interest, and wherein said ratio indicates the presence or absence of a fetal chromosomal abnormality. Claims 2-4, 8, 52, 56-57, 181-188, and 207-208 all depend

(directly or indirectly) from independent claim 1. Claim 152, as amended, is directed to a method for detecting the presence or absence of a fetal chromosomal abnormality, comprising both (a) determining the sequence of alleles of a locus of interest from template DNA, wherein the template DNA comprises a mixture of fetal DNA and maternal DNA, and wherein the template DNA is from a sample from a pregnant female, and (b) quantitating a ratio of the relative amount of the alleles in the mixture of fetal DNA and maternal DNA at a heterozygous locus of interest in the mixture that was identified by step (a), and wherein said ratio indicates the presence or absence of a fetal chromosomal abnormality. Claims 202-205 depend (directly or indirectly) from independent claim 152.

To establish a prima facie case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper prima facie case of obviousness since Umansky et al. in view of Saiki et al. does not teach or suggest a method comprising all of the elements of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208. Umansky et al. disclose a method of detecting specific fetal nucleic acid sequences by analyzing maternal urine. Saiki et al. teach a method of diagnosing sickle cell anemia by obtaining a patient sample, and determining the relative amount of alleles in a homogeneous sample, and expressing the results of the assay as a ratio. The combination of Umansky et al. and Saiki et al. does not teach all elements of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 because neither Umansky et al. nor Saiki et al. teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA at a heterozygous locus of interest.

The Examiner states that Umanksy et al. is silent regarding how to express the alleles. However, the Examiner asserts that "Saiki et al do teach a method diagnosing sickle cell anemia wherein the relative amount of the alleles at a heterozygous locus of interest are determined and results of the assay are expressed as a ratio." However, Saiki et al. does not teach or suggest "quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA." The ratio discussed in Saiki et al. is calculated using a homogenous DNA sample, and the ratio calculated represents alleles from a single individual. In contrast, the methods taught herein

comprise calculating a ratio of the relative amount of alleles in a heterogeneous mixture of DNA, i.e, maternal DNA and fetal DNA. The methods taught in the present invention comprise determining a ratio, wherein the ratio represents alleles from both the mother and the fetus. Umansky et al. and Saiki *et a*l. fail to teach or suggest this element, either alone or in combination.

Applicants have invented a method for detecting the presence or absence of a fetal chromosomal abnormality, wherein the method comprises, inter alia, quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. One such example, which is not to be construed as limiting the invention in any manner, can be found in Example 14 of the specification. As discussed in paragraphs [1018] through [1021], ratios were calculated at both chromosomes 13 and 21 in a heterogeneous mixture of 75% Down syndrome DNA and 25% maternal DNA. Single nucleotide polymorphisms were analyzed wherein the maternal genome was homozygous for one allele at a specific genetic site and the Down syndrome DNA was heterozygous at the same genetic site. If at a certain site, the maternal genome contains an adenine at both copies of chromosome 13, and the Down syndrome genome is comprised of one chromosome with an adenine nucleotide and one chromosome with a guanine nucleotide, then the ratio of G:A is 0.60 (0.75 (Down syndrome G allele)/(0.75 Down syndrome A allele + 0.25 + 0.25 (maternal A alleles).

On the other hand, if at a certain genetic site on chromosome 21, the maternal genome contains an adenine at both copies of chromosome 21, and the Down syndrome genome is comprised of two chromosome with an adenine nucleotide and one chromosome with a guanine nucleotide, then the ratio of G:A is 0.375 (0.75 (Down syndrome G allele)/(0.75 Down syndrome A allele + 0.75 Down syndrome A allele + 0.25 + 0.25 (maternal A alleles). Thus, the methods described in the present application detect chromosomal abnormalities using a method that comprises, inter alia, quantitating a ratio of alleles in a heterogeneous mixture of DNA, wherein the ratio represents alleles from more than one individual. This element is neither taught nor suggested by Umnasky *et al.*, either alone or in combination with Saiki *et al.*

Since Umansky et al. in view of Saiki et al. does not teach or suggest each and every element of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208, Applicant respectfully requests that the rejection of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 under 35 U.S.C. § 103 be withdrawn.

*Claims 5-6, 20-24, 26-30, 32-34, 36-39, 41 and 44-51:* The Office has rejected claims 5-6, 20-24, 26-30, 32-34, 36-39, 41 and 44-51 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Saiki *et al.* (NEJM 319: 537-541 (1988)] as applied against Claim 1 and further in view of Jones *et al.* [US 2003/0082576]. Applicant respectfully traverses this rejection.

Claims 5-6, 20-24, 26-30, 32-34, 36-39, 41, and 44-51 all depend (directly or indirectly) from independent claim 1 as described above. Claims 5-6, 20-24, 26-30, 32-34, 36-39, 41 and 44-51 are all generally directed to methods for detecting the absence or presence of a fetal chromosomal abnormality comprising calculating a ratio of the relative amount of alleles in a mixture of maternal and fetal DNA.

As discussed above, to establish a prima facie case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper prima facie case of obviousness since Umansky *et al.* in view of Saiki *et al.*, as applied against claim 1, and further in view of Jones *et al.* does not teach or suggest a method comprising all of the elements of claims 5-6, 20-24, 26-30, 32-34, 36-39, 41, and 44-51. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Saiki *et al.*, neither Umansky *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Jones *et al.*, either alone or in combination with Umanksy *et al.* or Saiki *et al.*

In addition, the Examiner states that "Jones et al do teach the exact assay recited in claims 20 and 21." Applicant respectfully traverses this assertion. Jones *et al.* discloses a method,

*inter alia*, which employs a solid substrate, and bridge amplification techniques, which include a solid substrate with bound first and second locus-specific primers. The method disclosed in Jones *et al.*, which relies on solid substrates, is quite distinct from the method recited in claim 20.

Claim 21 recites a method that comprises *inter alia* incorporating nucleotides into the digested DNA of (b), wherein (i) a nucleotide that terminates elongation, and is complementary to an allele of the locus of interest is incorporated into the 5' overhang of said allele, and (ii) a nucleotide complementary to a different allele of the locus of interest is incorporated into the 5' overhang of said different allele, and said terminating nucleotide, which is complementary to a nucleotide in the 5' overhang of said different allele, is incorporated into the 5' overhang of said different allele. Jones *et al.* fails to teach or suggest this element, and thus, the method recited in claim 21 is distinct from the method disclosed in Jones *et al.*

Since Umanksy *et al.* in view of Saiki *et al.* as applied against claim 1, and further in view of Jones *et al.*, does not teach or suggest each and every element of claims 5-6, 2-0-24, 26-30, 32-34, 36-39, 41, and 44-51, Applicant respectfully submits that the rejection of claims 5-6, 2-0-24, 26-30, 32-34, 36-39, 41, and 44-51 under 35 U.S.C. § 103 be withdrawn.

*Claims 9-12, 14-16, 18-19 and 189*: The Office has rejected claims 9-12, 14-16, 18-19 and 189 as being unpatentable over Umansky *et al.* (US 2002/0119478 (2002)) in view of Saiki *et al.* (NEJM 319: 537-541 (1988)) as applied against claim 4 above and further in view of Kiessling (US Patent No. 5,618,664). Applicant respectfully traverses this rejection.

Claims 9-12, 14-16, 18-19 and 189 depend from independent claim 1, as amended, and therefore incorporates all elements of claim 1 as described above. In addition, claims 9-12 and 189 further are directed to the addition of agents to inhibit cell lysis. Claims 14-16 further are directed to sources of the template DNA. Claims 18-19 are directed to methods wherein prior to analysis of the template DNA, maternal DNA is sequenced to identify homozygous or heterozygous loci of interest, respectively.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Umansky *et. al*, alone or in combination with Saiki *et al.* as applied against claim 4, and further in view of Kiessling does not teach or suggest all elements of claims 9-12, 14-16, 18-19 and 189. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Saiki *et al.*, neither Umansky *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Kiessling, either alone or in combination with Umanksy *et al.* or Saiki *et al.* For a more detailed explanation of the deficiencies of the Kiessling reference, please see pages 33-35 of the present response.

Since Umanksy *et al.* in view of Saiki *et al.* as applied against claim 4 and further in view of Kiessling does not teach or suggest each and every element of claims 9-12, 14-16, 18-19 and 189, Applicant respectfully submits that the rejection of claims 9-12, 14-16, 18-19 and 189 under 35 U.S.C. § 103 be withdrawn.

*Claim 43*: The Office has rejected claim 43 under 35 U.S.C. § 103(a) as being unpatentable over Umansky *et al.* (US 2002/0119478) in view of Saiki *et al.* (NEJM 319: 537-541 (1988)) and Jones *et al.* (US2003/0082576) as applied against Claims 20 and 21 above and further in view of MacLeod *et al.* (US 6,221,600) and Polisson (US 5098,839). Applicant respectfully traverses this rejection.

Claim 43 depends indirectly from claim 1, as amended, and therefore incorporates all elements of claim 1 as described above. In addition, claim 43 recites specific restriction enzymes useful in the claimed method.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Umansky *et al.*, alone or in combination with Saiki *et al.* and/or Jones *et al.* and/or MacLeod *et al.* and/or Polisson

does not teach or suggest all elements of claim 43. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Saiki *et al.*, neither Umansky *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Jones *et al.* and/or MacLeod *et al.* and/or Polisson, either alone or in combination with Umanksy *et al.* or Saiki *et al.* As discussed above, Jones *et al.* fails to teach or suggest the methods recited in claims 20 and 21.

Since Umanksy *et al.* in view of Saiki *et al.* and Jones *et al.* as applied against claims 20 and 21 and further in view of MacLeod *et al.* and Polisson does not teach or suggest each and every element of claim 43, Applicant respectfully submits that the rejection of claim 43 under 35 U.S.C. § 103 be withdrawn.

*Claims 58-68, 87-102, 190-196 and 201*: The Office has rejected claims 58-68, 87-102, 190-196 and 201 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Kiessling [US Patent No. 5,618,664]. Applicant respectfully traverses this rejection.

Amended claim 58 recites a method comprising determining the sequence of a locus of interest on free fetal DNA isolated from a sample obtained from a pregnant female, wherein said sample comprises free fetal DNA and an agent that inhibits lysis of cells, if cells are present, wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor. Claims 59-68, and 190-196 all depend (directly or indirectly) from claim 58. Amended claim 87 recites a method for preparing a sample for analysis comprising isolating free fetal nucleic acid from the sample, wherein said sample comprises an agent to inhibit the lysis of cells, if cells are present, and wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor. Claims 88-102, and 201 all depend (directly or indirectly) from independent claim 87.

The factors a Court considers when determining obviousness and non-obviousness in the United States were outlined by the Supreme Court in Graham et al. v. John Deere Co. of Kansas City et al., 383 U.S. 1 (1966) and are commonly referred to as the "Graham factors." The Court held that obviousness should be determined by looking at: (1) the scope and content of the prior art; (2) the level of ordinary skill in the prior art; (3) the difference between the claimed invention and the prior art; and (4) objective evidence of non-obviousness. In addition, the Court outlined several factor that show "objective evidence of non-obviousness" and include commercial success, long-felt need but unsolved needs, and failure of others. In a recent Supreme Court decision, KSR v. Teleflex, the Court re-affirmed the continuing validity of its decision in Graham as the touchstone for an obviousness analysis, stating "there is no necessary inconsistency between the idea underlying the TSM [teaching, suggestion, motivation] test and the Graham analysis" (slip opinion at 15).

Methods for determining the sequence of a locus of interest on free fetal DNA and for preparing a sample for analysis comprising free fetal DNA serve a long felt need in the medical community. Available protocols for prenatal diagnosis have limitations. Screening tests, such as nuchal translucency and the quadruple screen are noninvasive, but diagnosis requires further invasive testing. Invasive diagnostic tests, such as amniocentesis and chorionic villus sampling (CVS), are approximately 99% accurate in identifying the spectrum of chromosomal abnormalities, but are associated with increased risks to the pregnancy.

Analysis of free fetal DNA in the maternal circulation provides an alternative to existing prenatal tests. However, the seemingly low percentage of free fetal DNA in the maternal circulation (initial studies reported a mean of only 3.4% free fetal DNA in the mid to late first trimester) has limited the clinical utility of free fetal DNA (Lo et al., Am. J. Hum. Genet., 62:768-773, 1998; Pertl and Bianchi, Obstetrics and Gynecology, Vol. 98, No.3: 483-490, 2001). The methods encompassed within claims 58-68, 87-102, 190-196 and 201 elevate this problem, and thus provide a solution to a long-felt need in the medical community.

Applicant has discovered that the addition of a cell lysis inhibitor to a sample prior to determining the sequence of a locus of interest on free fetal DNA can significantly and unexpectedly increase the proportion of fetal DNA versus maternal DNA obtained from a sample such as a plasma sample obtained from the blood of a pregnant woman. Examples of the impact of using a cell lysis inhibitor such as formalin to inhibit cell lysis prior to determining the sequence of a locus of interest on free fetal DNA are provided in Example 4 of Applicant's specification. As shown in Example 4, the amounts of fetal DNA isolated from maternal blood samples were significantly, and unexpectedly, higher for samples treated with formalin. As indicated in paragraph [0490] in example 4, in one set of experiments, the percentage of fetal DNA present in the sample without formalin was 1.56%, whereas the percentage of fetal DNA in the sample treated with formalin was 25%. The high percentage of fetal DNA versus maternal DNA, which can be obtained from the plasma of maternal blood to which cell lysis inhibitor (*e.g.* formalin) has been added is further demonstrated in Example 15 of Applicant's specification (see, *e.g.*, Table XXI).

Furthermore, the Manual for Patent Examining Procedure states that "greater than expected results are evidence of non-obviousness." *See* MPEP 716.02(a). As discussed above, prior to the Applicants' work, the mean percentage of free fetal DNA in a maternal sample was expected to be about 3%. However, using the methods encompassed within claims 58-68, 87-102, 190-196 and 201, the mean percentage is unexpectedly and significantly increased to 25%. Thus, the methods encompassed within claims 58-68, 87-102, 190-196 and 201 produce unexpected results, and therefore, the claimed methods would not have been obvious to one of ordinary skill in the art.

The Examiner asserts that one of ordinary skill in the art would have been motivated to combine the teachings of Umansky *et al.* with the teachings of Kiessling. Applicant respectfully submits that there is no motivation to combine the teachings of the reference Umansky *et al.* with the teachings of the reference Kiessling. One of ordinary skill in the art would not be motivated to combine the teachings of Kiessling with Umansky as the DNA analyzed in the two methods is quite distinct (*i.e*, DNA in Umansky *et al.* is free and circulating outside of a cell, while the DNA analyzed in Kiessling is in and/or is released from a fixed cell). Umansky *et al.* disclose a method

that analyzes free fetal DNA in a maternal urine sample. Conversely, the methodology disclosed in Kiessling "fixes" leukocytes or other cells, and subsequently DNA is isolated from the fixed leukocytes or other cells (see, e.g., col. 10, lines 50-54) or analyzed in the cells. The DNA analyzed in Umansky *et al.* is free nucleic acid, which has already been released from the cell, while the DNA in Kiessling is contained within a fixed isolated cell. Thus, one of ordinary skill in the art would not have been motivated to combine the disclosures of Umanksy *et al.* and Kiessling.

Furthermore, one of ordinary skill in the art would not be motivated to combine the Kiessling disclosure with that of Umansky *et al.* because the biological sample used in Umansky is urine, which is considered to be sterile, and Kiessling discloses a method to reduce the risk of exposure to infectious agents. According to Kiessling, "the present invention overcomes the problems inherent in the prior art by providing methods for handling biological fluid samples to reduce the exposure of health care personnel to infectious agents, while preserving the analytes contained therein for analysis." *See* col 2, line 64 through col. 3, line 1. However, the biological sample disclosed in Umansky *et al.* is urine. According to the National Kidney and Urologic Diseases Information Clearinghouse, "normally, urine is sterile." (see article entitled "Urinary Tract Infections in Adults," available at http://kidney.niddk.nih.gov/kudiseases/pubs/utiadult) Thus, one of ordinary skill in the art would not be motivated to combine the disclosures of Umanksy *et al.* and Kiessling because there is no risk of exposure to infectious agents when working with the biological sample disclosed in Umanksy *et al.* When urine is the biological sample as in Umansky *et al.*, there is no motivation to practice the teachings of Kiessling because urine is considered sterile and thus, there is no risk of exposure to infectious agents.

For instance, Kiessling specifically mentions that her method can be used to reduce the risk of exposure to HIV. However, according to an HIV Tutorial provided by the University of Utah Medical School, "the appearance of HIV in saliva, ***urine***, tears, and sweat is of no major clinical importance, as transmission of HIV through these fluids does not routinely occur." *See* document entitled "HIV Tutorial" from the University of Utah Medical School, available at http://library.med.utah.edu/WebPath/TUTORIAL/AIDS/HIV.HTML, at page 4. Therefore, one of

ordinary skill in the art practicing the methods of Umanksy *et al.* would not be motivated to include the methods disclosed in Kiessling.

Furthermore, even assuming one of ordinary skill in the art did want to reduce the risk of exposure to infectious agents when working with urine, there are numerous methods available to achieve this goal, and there is no motivation to choose the method disclosed by Kiessling. For example, U.S. Patent No. 5,985,260 discloses a method of disinfecting blood and blood components comprising preparing and immediately adding active albumin-iodine complex to the material to be disinfected. In addition, Moreton and Delves report that the addition of Virkon, which is a viral disinfectant, to clinical samples can reduce the likelihood of infection (Moreton and Delves, J. Anal. At. Spectrom, 1999, 14:893-894). Thus, even if one of ordinary skill in the art was concerned with reducing the risk of exposure to infections agents in a urine sample, there are numerous methods available to one of ordinary skill in the art and there is nothing that would lead one of ordinary skill to the disclosure of Kiessling.

The methods disclosed in claims 58-68, 87-102, 190-196 and 201 serve a long-felt need in the medical community, and provide unexpected results, and are therefore non-obvious. In addition, there is no motivation to combine the disclosures of Umanksy *et al.* and Kiessling, thus, Applicant respectfully requests that the rejection of claims 58-68, 87-102, 190-196 and 201 under 35 U.S.C. § 103 be withdrawn.

*Claims 69-70 and 83*: The Office has rejected claims 69-70 and 83 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Kiessling [US Patent No. 5,618,664] as applied above against Claim 58 and further in view of Saiki *et al.* (NEJM 319: 537-541 (1988)]. Applicant respectfully traverses this rejection.

Claims 69, 70 and 83 depend from amended claim 58 as discussed above. Claim 69 further defines said locus of interest as a single nucleotide polymorphism. Claim 70 further defines said locus of interest as a mutation. Claim 83 recites specified methods for defining the locus of interest.

As discussed above with respect to the rejection of claims 58-68, 87-102, 190-196 and 201 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Kiessling, the methods encompassed in claims 69, 70 and 83 produce unexpected results and serve a long-felt need in the medical community, and thus, the methods recited in claims 69, 70 and 83 are non-obvious. In addition, to establish a prima facie case of obviousness, there must be some suggestion or motivation to modify the reference or to combine reference teachings. Applicant respectfully submits that there is no motivation to combine the teachings of the reference Umansky *et al.* with the teachings of the reference Kiessling as applied against claim 58 and further in view of Saiki *et al.* because, *inter alia*, the methods disclosed in Umanksy *et al.* encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA. Furthermore, the methods of Kiessling are concerned with reducing the risk of exposure to infectious agents while the methods disclosed by Umanksy *et al.* use urine as the biological sample, which is considered sterile, and thus there would be no motivation to combine the disclosures of Umanksy *et al.* and Kiessling. Saiki et al. does not teach or suggest the claimed methods or provide any motivation to combine the disclosures of Umansky *et al.* and Kiessling.

Since the methods recited in claims 69, 70 and 83 produce unexpected results and serve a long-felt need in the medical community, and since there is no motivation to combine Umanksy *et al.* and Kiessling as applied against claim 58 and Saiki *et al.*, alone or in combination, does not teach or suggest the claimed methods, Applicant respectfully requests that the rejection of claims 69-70 and 83 under 35 U.S.C. § 103 be withdrawn.

*Claims 71-82*: The Office rejected claims 71-82 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Kiessling [US Patent No. 5,618,664] as applied against claim 58 above and further in view of Jones et al. [US 2003/0082576]. Applicant respectfully traverses this rejection.

Claims 71-82 all depend (directly or indirectly) from claim 58 as discussed above. Claim 71 recites that the sequences of multiple loci of interest are determined. Claim 72 depends

from claim 71 and recites that the loci of interest are on multiple chromosomes. Claims 73 and 74 recite methods for determining the sequence, and claims 75-82 recite additional elements for determining the sequence of the locus of interest.

As discussed above with respect to the rejection of claims 58-68, 87-102, 190-196 and 201 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Kiessling, the methods recited in claims 71-82 produce unexpected results and serve a long-felt need in the medical community, and thus are non-obvious. In addition, Applicant respectfully submits that there is no motivation to combine the teachings of the reference Umansky *et al.* with the teachings of the reference Kiessling as applied against claim 58 and further in view of Jones *et al.* because, *inter alia*, the methods disclosed in Umanksy *et al.* encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA. In addition, the methods of Kiessling are concerned with reducing the risk of exposure to infectious agents while the methods disclosed by Umanksy *et al.* use urine as the biological sample, which is considered sterile, and thus there would be no motivation to combine the disclosures of Umanksy *et al.* and Kiessling. Jones *et al.* does not teach or suggest the claimed methods or provide any motivation to combine the disclosures of Umansky *et al.* and Kiessling.

Since the methods recited in claims 71-82 produce unexpected results and serve a long-felt need in the medical community, and since there is no motivation to combine Umanksy *et al.* and Kiessling as applied against claim 58 and since Jones *et al.*, alone or in combination, does not teach or suggest the claimed invention, Applicant respectfully requests that the rejection of claims 71-82 under 35 U.S.C. § 103 be withdrawn.

*Claims 132, 134-142*: The Office has rejected claims 132, and 134-142 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Saiki *et al.* (NEJM 46(2): 301-302 (2000)] as applied against Claim 1 above and further in view of Chen *et al.* [Genome Research 10: 549-557 (2000)]. Applicant respectfully traverses this rejection.

Claims 132 and 134-142 depend (directly or indirectly) from amended claim 1 as discussed above. Claim 132 further defines the method used to determine the sequence of the locus of interest. Claims 134-142 recite further elements of the method used to determine the sequence of the locus of interest.

As already discussed, to establish a prima facie case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper prima facie case of obviousness since Umansky *et al.*, alone or in combination with Saiki *et al.* and/or Chen *et al.* does not teach or suggest a method comprising all of the elements of claims 132, and 134-142. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Saiki *et al.*, neither Umansky *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Chen *et al.*, either alone or in combination with Umanksy *et al.* or Saiki *et al.*

Since Umansky *et al.* in view of Saiki *et al.* as applied against claim 1 and since Chen *et al.*, alone or in combination, does not teach or suggest each and every claim element of claims 132, and 134-142, Applicant respectfully requests that the rejection of claims 132, and 134-142 under 35 USC 103 be withdrawn.

*Claims 133-142 and 206:* The Office has rejected claims 133-142 and 206 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Kiessling [US Patent No. 5,618,664] as applied against Claim 58 above and further in view of Chen *et al.* [Genome Research 10: 549-557 (2000)]. Applicant respectfully traverses this rejection.

Claims 133-142 and 206 depend (directly or indirectly) from amended claim 58 as discussed above. Claim 133 defines a method for determining the sequence of the locus of interest.

Claims 134-142 further recite elements of the method used to determine the sequence of the locus of interest. Claim 206 recites that the agent added to inhibit cell lysis is a cell lysis inhibitor.

As discussed above with respect to the rejection of claims 58-68, 87-102, 190-196 and 201 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Kiessling, the methods recited in claims 133-142 and 206 produce unexpected results and serve a long-felt need in the medical community, and thus are non-obvious. In addition, Applicant respectfully submits that there is no motivation to combine the teachings of the reference Umansky *et al.* with the teachings of the reference Kiessling as applied against claim 58, and further in view of Chen *et al.* because, *inter alia*, the methods disclosed in Umanksy *et al.* encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA. In addition, the methods of Kiessling are concerned with reducing the risk of exposure to infectious agents while the methods disclosed by Umanksy *et al.* use urine as the biological sample, which is considered sterile, and thus there would be no motivation to combine the disclosures of Umanksy *et al.* and Kiessling. Chen *et al.* does not teach or suggest the claimed methods or provide any motivation to combine the disclosures of Umansky *et al.* and Kiessling.

Since the methods recited in claims 133-142 and 206 produce unexpected results and serve a long-felt need in the medical community, and since there is no motivation to combine Umanksy *et al.* and Kiessling as applied against claim 58, and since Chen *et al.* does not teach each and every element of the claims or provide motivation to combine Umansky *et al.* and Kiessling, Applicant respectfully requests that the rejection of claims 133-142 and 206 under 35 U.S.C. § 103 be withdrawn.

*Claims 143 and 145-146*: The Office has rejected claims 143 and 145-146 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Saiki *et al.* (NEJM 46(2): 301-302] as applied against Claim 1 above and further in view of Livak *et al.* [U.S. Patent No. 5,538,848]. Applicant respectfully traverses this rejection.

Claims 143 and 145-146 depend (directly or indirectly) from amended claim 1 as discussed above. Claim 143 defines a method used to determine the sequence of the locus of interest. Claims 145 and 146 recite further elements of the method used to determine the sequence of the locus of interest.

As previously discussed, to establish a prima facie case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper prima facie case of obviousness since Umansky *et al.*, alone or in combination with Saiki *et al.* and/or Livak *et al.*, does not teach or suggest a method comprising all of the elements of claims 143, and 145-146. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Saiki *et al.*, neither Umansky *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Livak *et al.*, either alone or in combination with Umanksy *et al.* or Saiki *et al.*

Since Umansky *et al.* in view of Saiki *et al.* as applied against claim 1 and since Livak *et al.*, alone or in combination, does not teach or suggest each and every element of claims 143 and 145-146, Applicant respectfully requests that the rejection of claims 143 and 145-146 under 35 U.S.C. § 103 be withdrawn.

*Claims 144-146 and 206*: The Office has rejected claims 144-146 and 206 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* [US 2002/0119478 (2002)] in view of Kiessling [US Patent No. 5,618,664 (1988)] as applied against Claim 58 above and further in view of Livak *et al.* [U.S. Patent No. 5,538,848 (1996)]. Applicant respectfully traverses this rejection.

Claims 144-146 and 206 all depend (directly or indirectly) from amended claim 58 as discussed above. Claims 144-146 define a method used to determine the sequence of the locus of interest. Claim 206 recites a particular type of agent used to prevent lysis of cells.

As discussed above with respect to the rejection of claims 58-68, 87-102, 190-196 and 201 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Kiessling, the methods encompassed within claims 144-146 and 206 produce unexpected results and serve a long-felt need in the medical community, and thus are non-obvious. In addition, Applicant respectfully submits that there is no motivation to combine the teachings of the reference Umansky *et al.* with the teachings of the reference Kiessling as applied against claim 58, and further in view of Livak *et al.* because, *inter alia*, the methods disclosed in Umanksy *et al.* encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA. In addition, the methods of Kiessling are concerned with reducing the risk of exposure to infectious agents while the methods disclosed by Umanksy *et al.* use urine as the biological sample, which is considered sterile, and thus there would be no motivation to combine the disclosures of Umanksy *et al.* and Kiessling. Livak *et al.* does not teach or suggest the claimed methods or provide any motivation to combine the disclosures of Umansky *et al.* and Kiessling

Since the methods recited in claims 144-146 and 206 produce unexpected results and serve a long-felt need in the medical community, and since there is no motivation to combine Umanksy *et al.* and Kiessling as applied against claim 58, and since Livak *et al.* does not teach each and every element of the claims or provide motivation to combine Umansky *et al.* and Kiessling, Applicant respectfully requests that the rejection of claims 144-146 and 206 under 35 U.S.C. § 103 be withdrawn.

*Claims 148-151*: The Office has rejected claims 148-151 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Saiki *et al.* (NEJM 46(2): 301-302 (2000)] and Chen *et al.* [Genome Research 10: 549-557 (2000)] as applied against Claim 132 above and further in view of Kiessling [US Patent No. 5,618,664 (1988)]. Applicant respectfully traverses this rejection.

Claims 148-151 all depend (indirectly) from amended claim 1 as discussed above. Claims 148-151 further recite that an agent that inhibits cell lysis is added to the sample.

As already discussed, to establish a prima facie case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper prima facie case of obviousness since Umansky *et al.*, alone or in combination with Saiki *et al.* and Chen *et al.* as applied against claim 132 and further in view of Kiessling does not teach or suggest a method comprising all of the elements of claims 148-151. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Saiki *et al.*, neither Umansky *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Chen *et al.* or Kiessling, either alone or in combination with Umanksy *et al.* or Saiki *et al.*

Since Umansky *et al.* in view of Saiki *et al.* and Chen *et al.* as applied against claim 132 and since Kiessling, alone or in combination, does not teach or suggest each and every claim element of claims 148-151, Applicant respectfully requests that the rejection of claims 148-151 under 35 U.S.C. § 103 be withdrawn.

*Claims 148-151*: The Office has rejected claims 148-151 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* [US 2002/0119478] in view of Saiki *et al.* (NEJM 46(2): 301-302 (2000)] as applied against Claim 143 above and further in view of Livak *et al.* [U.S. Patent No. 5,538,848] as applied against Claim 143 above and further in view of Kiessling [US Patent No. 5,618,664]. Applicant respectfully traverses this rejection.

Claims 148-151 all depend (indirectly) from amended claim 1 as discussed above. Claims 148-151 further recite that an agent that inhibits cell lysis is added to the sample.

As already discussed, to establish a prima facie case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper prima facie case of obviousness since Umansky *et al.*, alone or in combination with Saiki *et al.* as applied against claim 143 and further in

view of Livak *et al.* and Kiessling does not teach or suggest a method comprising all of the elements of claims 148-151. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-188, 202-205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Umansky *et al.* in view of Saiki *et al.*, neither Umansky *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Livak *et al.* and Kiessling, either alone or in combination, with Umanksy *et al.* or Saiki *et al.*

Since Umansky *et al.* in view of Saiki *et al.* as applied against claim 143 and further in view of Livak *et al.* and Kiessling does not teach or suggest each and every claim element of claims 148-151, Applicant respectfully requests that the rejection of claims 148-151 under 35 U.S.C. § 103 be withdrawn.

*Claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208*: The Office has rejected claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)]. Applicant respectfully traverses this rejection.

Amended claims 1 and 152 are discussed above. Claims 2-4, 8, 52, 56-57, 181-183, 185-187, and 207-208 all depend (directly or indirectly) from amended claim 1. Claims 202-205 depend (directly or indirectly) from independent claim 152.

To establish a prima facie case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper prima facie case of obviousness since Amicucci et al. in view of Saiki et al. does not teach or suggest a method comprising all of the elements of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208. Amicucci et. al discloses a method using maternal plasma for prenatal diagnosis of myotonic dystrophy (DM) by monitoring the pregnancy of an unaffected woman whose husband was affected by DM. Saiki et al. teach a method of diagnosing sickle cell anemia by obtaining a patient sample, and determining the relative amount

of alleles, and expressing the results of the assay as a ratio. The combination of Amicucci et. al and Saiki et al. does not teach all elements of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 because neither Amicucci et. al nor Saiki et al. teach or suggest calculating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA.

The Examiner states that Amicucci et. al does not teach expressing the relative amount of alleles as a ratio. However, the Examiner asserts that "Saiki et al. do teach a method diagnosing sickle cell anemia wherein the relative amount of the alleles at a heterozygous locus of interest are determined and results of the assay are expressed as a ratio." However, Saiki et al. does not teach or suggest "quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA." The ratio discussed in Saiki et al. is calculated using a homogenous DNA sample, and the ratio calculated represents alleles from a single individual. In contrast, the methods taught herein comprise calculating a ratio of the relative amount of alleles in a heterogeneous mixture of DNA, i.e, maternal DNA and fetal DNA. The methods taught in the present invention comprise determining a ratio, wherein the ratio represents alleles from both the mother and the fetus. This element is not taught or suggested by the combination of Amicucci et al. and Saiki et al.

Applicants have invented a method for detecting the presence or absence of a fetal chromosomal abnormality, wherein the method comprises, inter alia, quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. One such example, which is not to be construed as limiting the invention in any manner, can be found in Example 14 of the specification. As discussed in paragraphs [1018] through [1021], ratios were calculated at both chromosomes 13 and 21 in a heterogeneous mixture of 75% Down syndrome DNA and 25% maternal DNA. Single nucleotide polymorphisms were analyzed wherein the maternal genome was homozygous for one allele and the Down syndrome DNA was heterozygous at the same genetic site. If at a certain site, the maternal genome contains an adenine at both copies of chromosome 13, and the Down syndrome genome is comprised of one chromosome with an adenine nucleotide at that genetic site and one chromosome with a guanine nucleotide, then the ratio of G:A is 0.60 (0.75 Down syndrome G allele /(0.75 Down syndrome A allele + 0.25 + 0.25 (maternal A alleles).

On the other hand, at chromosome 21, the expected ratio would be 0.375. If at a certain site, the maternal genome contains an adenine at both copies of chromosome 21, and the Down syndrome genome is comprised of two chromosome with an adenine nucleotide at that genetic site and one chromosome with a guanine nucleotide, then the ratio of G:A is 0.375 (0.75 Down syndrome G allele/(0.75 Down syndrome A allele + 0.75 Down syndrome A allele + 0.25 + 0.25 (maternal A alleles). Thus, the methods described in the present application detect chromosomal abnormalities using a method that comprises, inter alia, quantitating a ratio of alleles in a heterogeneous mixture of DNA, wherein the ratio represents alleles from more than one individual. This element is neither taught nor suggested by Amicucci et al., either alone or in combination with Saiki et al.

Since Amicucci et. al in view of Saiki et al. does not teach or suggest each and every element of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208, Applicant respectfully requests that the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 USC § 103 be withdrawn.

*Claims 184, 188 and 204*: The Office has rejected claims 184, 188 and 204 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] as applied against Claims 182, 186 and 203 above and further in view of Lo *et al.* [The Lancet 350: 485-487 (1997)]. Applicant respectfully traverses this rejection.

Claims 184 and 188 depend indirectly from amended claim 1 discussed above. Claims 184 and 188 recite sources of the template DNA. Claim 204 depends from amended claim 152, and further recites a source of the template DNA.

To establish a *prima facie* case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper *prima facie* case of obviousness since Amicucci *et al.* in view of Saiki *et al.* as applied against Claims 182, 186 and 203 above and further in view of Lo *et. al* does

not teach or suggest a method comprising all elements of claims 184, 188 and 204. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Lo *et al.*, either alone or in combination with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et. al* in view of Saiki *et al.*, and since Lo *et al.*, alone or in combination, does not teach or suggest each and every element of claims 184, 188 and 204, Applicant respectfully requests that the rejection of claims 184, 188 and 204 under 35 USC § 103 be withdrawn.

*Claims 5-6, 20-24, 26-30, 32-34, 36-39, 41, 44-52 and 56-57*: The Office has rejected claims 5-6, 20-24, 26-30, 32-34, 36-39, 41, 44-52 and 56-57 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] as applied against Claim 1 above and further in view of Jones et al. [US 2003/0082576]. Applicant respectfully traverses this rejection.

Claims 5-6, 20-24, 26-30, 32-34, 36-39, 41, 44-52 and 56-57 all depend (directly or indirectly) from amended claim 1 as discussed above. Claims 5-6, 20-24, 26-30, 32-34, 36-39, 41 and 44-52 and 56-57 all are generally directed to methods for detecting the absence or presence of a fetal chromosomal abnormality comprising calculating a ratio of the relative amount of alleles in a mixture of maternal and fetal DNA.

As discussed above, to establish a prima facie case of obviousness, the cited references must teach or suggest each and every claim limitation. Applicant respectfully submits that the Office has not met its burden of establishing a proper prima facie case of obviousness since Amicucci *et al.* in view of Saiki *et al.*, as applied against claim 1, and further in view of Jones *et al.* does not teach or suggest a method comprising all of the elements of claims 5-6, 20-24, 26-30, 32-

34, 36-39, 41 and 44-52 and 56-57. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Jones *et al.*, either alone or in combination with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et al.* in view of Saiki *et al.* as applied against claim 1, and further in view of Jones *et. al*, does not teach or suggest each and every element of claims 5-6, 20-24, 26-30, 32-34, 36-39, 41 and 44-52 and 56-57, Applicant respectfully submits that the rejection of claims 5-6, 2-0-24, 26-30, 32-34, 36-39, 41, and 44-52 and 56-57 under 35 U.S.C. § 103 be withdrawn.

*Claims 9-12, 14-15, 18-19 and 189:* The Office has rejected claims 9-12, 14-15, 18-19 and 189 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] as applied against Claim 4 above and further in view of Kiessling [US Patent No. 5,618,664]. Applicant respectfully traverses this rejection.

Claims 9-12, 14-15, 18-19 and 189 depend from amended claim 1 and therefore incorporates all elements of claim 1 as described above. In addition, claims 9-12 and 189 further are directed to the addition of agents to inhibit cell lysis. Claims 14-15 further are directed to sources of the template DNA. Claims 18-19 further are directed to methods wherein prior to analysis of the template DNA, maternal DNA is sequenced to identify homozygous and heterozygous loci of interest, respectively.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Amicucci *et. al*, alone or in combination with Saiki *et al.* as applied against claim 4, and further in view of Kiessling does not teach or suggest all elements of claims 9-12, 14-15, 18-19 and 189. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and

207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Kiessling, either alone or in combination with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et al.* in view of Saiki *et al.* as applied against claim 4 and further in view of Kiessling does not teach or suggest each and every element of claims 9-12, 14-15, 18-19 and 189, Applicant respectfully submits that the rejection of claims 9-12, 14-15, 18-19 and 189 under 35 U.S.C. § 103 be withdrawn.

*Claims 16, 18 and 19*: The Office has rejected claims 16, 18 and 19 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] and Kiessling [US Patent No. 5,618,664 (1988)] as applied against Claim 12 above and further in view of Lo et al. [The Lancet 350: 485-487 (1997)]. Applicant respectfully traverses this rejection.

Claims 16, 18 and 19 all depend indirectly from amended claim 1 discussed above. Claims 16 recites a source of the template DNA and claims 18 and 19 recite features of the locus of interest.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Amicucci *et. al*, alone or in combination with Saiki *et al.* and Kiessling as applied against claim 12, and further in view of Lo *et al.* does not teach or suggest all elements of claims 16 and 18-19. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Kiessling or Lo *et al.*, either alone or in combination with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et al.* in view of Saiki *et al.* and Kiessling as applied against claim 12 and further in view of Lo *et al.* does not teach or suggest each and every element of claims 16 and 18-19, Applicant respectfully submits that the rejection of claims 16 and 18-19 under 35 U.S.C. § 103 be withdrawn.

*Claim 43*: The Office has rejected claim 43 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] and Jones *et al.* [US 2003/0082576] as applied against Claims 20 and 21 above and further in view of MacLeod *et al.* [U.S. Patent No. 6,221,600] and Polisson [US 5,098,839]. Applicant respectfully traverses this rejection.

Claim 43 depends indirectly from amended claim 1, and therefore incorporates all elements of claim 1 as described above. In addition, claim 43 recites specific restriction enzymes that can be in the claimed method for determining the sequence of alleles.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Amicucci *et al.*, alone or in combination with Saiki *et al.* and/or Jones *et al.* and/or MacLeod *et al.* and/or Polisson does not teach or suggest all elements of claim 43. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Jones *et al.* and/or MacLeod *et al.* and/or Polisson, either alone or in combination, with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et al.* in view of Saiki *et al.* and Jones *et al.* as applied against claims 20 and 21 and further in view of MacLeod *et al.* and Polisson does not teach or suggest each and every element of claim 43, Applicant respectfully submits that the rejection of claim 43 under 35 U.S.C. § 103 be withdrawn.

Claims 58-65, 67-68, 87-102, 190-194, 196 and 201: The Office has rejected claims 58-65, 67-68, 87-102, 190-194, 196 and 201 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Kiessling [US Patent No. 5,618,664]. Applicant respectfully traverses this rejection.

Amended claim 58 and 87 are discussed above. Claims 59-65, 67-68, 190-194 and 196 all depend (directly or indirectly) from claim 58. Claims 88-102, and 201 all depend (directly or indirectly) from independent claim 87.

The factors a Court considers when determining obviousness and non-obviousness in the United States were outlined by the Supreme Court in *Graham et al. v. John Deere Co. of Kansas City et al.*, 383 U.S. 1 (1966) and are commonly referred to as the "Graham factors." The Court held that obviousness should be determined by looking at: (1) the scope and content of the prior art; (2) the level of ordinary skill in the prior art; (3) the difference between the claimed invention and the prior art; and (4) objective evidence of non-obviousness. In addition, the Court outlined several factors that show "objective evidence of non-obviousness" and include commercial success, long-felt need but unsolved needs, and failure of others. In a recent Supreme Court decision, *KSR v. Teleflex*, the Court re-affirmed the continuing validity of its decision in *Graham* as the touchstone for an obviousness analysis, stating "there is no necessary inconsistency between the idea underlying the TSM test and the Graham analysis" (slip opinion at 15).

Methods for determining the sequence of a locus of interest on free fetal DNA and for preparing a sample for analysis comprising free fetal DNA serve a long felt need in the medical community. Available protocols for prenatal diagnosis have limitations. Screening tests, such as nuchal translucency and the quadruple screen are noninvasive, but diagnosis requires further invasive testing. Invasive diagnostic tests, such as amniocentesis and chorionic villus sampling (CVS), are approximately 99% accurate in identifying the spectrum of chromosomal abnormalities, but are associated with increased risks to the pregnancy.

Analysis of free fetal DNA in the maternal circulation provides an alternative to existing prenatal tests. However, the seemingly low percentage of free fetal DNA in the maternal circulation (initial studies reported a mean of only 3.4% free fetal DNA in the mid to late first trimester) has limited the clinical utility of free fetal DNA. The methods encompassed within claims 58-65, 67-68, 87-102, 190-194, 196 and 201 elevate this problem, and thus provide a solution to a long-felt need in the medical community.

Applicant has discovered that the addition of a cell lysis inhibitor to a sample prior to determining the sequence of a locus of interest on free fetal DNA can significantly and unexpectedly increase the proportion of fetal DNA versus maternal DNA obtained from a sample such as a plasma sample obtained from the blood of a pregnant woman. Examples of the impact of using a cell lysis inhibitor such as formalin to inhibit cell lysis prior to determining the sequence of a locus of interest on free fetal DNA are provided in Example 4 of Applicant's specification. As shown in Example 4, the amounts of fetal DNA isolated from maternal blood samples were significantly, and unexpectedly, higher for samples treated with formalin. As indicated in paragraph [0490] in example 4, in one set of experiments, the percentage of fetal DNA present in the sample without formalin was 1.56%, whereas the percentage of fetal DNA in the sample treated with formalin was 25%. The high percentage of fetal DNA versus maternal DNA, which can be obtained from the plasma of maternal blood to which cell lysis inhibitor (*e.g.* formalin) has been added is further demonstrated in Example 15 of Applicant's specification (see, *e.g.*, Table XXI).

Furthermore, the Manual for Patent Examining Procedure states that "greater than expected results are evidence of non-obviousness." *See* MPEP 716.02(a). As discussed above, prior to the Applicants' work, the mean percentage of free fetal DNA in a maternal sample was expected to be about 3%. However, using the methods encompassed within claims 58-65, 67-68, 87-102, 190-194, 196 and 201, the mean percentage is unexpectedly and significantly increased, as demonstrated in Example 4. Thus, the methods encompassed within claims 58-65, 67-68, 87-102, 190-194, 196 and 201 produce unexpected results, and therefore, the claimed methods would not have been obvious to one of ordinary skill in the art.

The Examiner asserts that one of ordinary skill in the art would have been motivated to combine the teachings of Amicucci *et al.* with the teachings of Kiessling. Applicant respectfully submits that there is no motivation to combine the teachings of the reference Amicucci *et al.* with the teachings of the reference Kiessling. As previously noted, the methods of Amicucci *et al.* are directed to methods for prenatal diagnosis of DM using maternal plasma. Amicucci *et al.* relies on the presence of free fetal DNA in the maternal plasma. Conversely, the methodology disclosed in Kiessling "fixes" leukocytes or other cells, and subsequently, DNA is isolated from the fixed leukocytes (see col. 10, lines 50-54) or analyzed in the cells. The DNA analyzed in Amicucci *et al.* is free nucleic acid, while the DNA in Kiessling is contained within a fixed isolated cell. It would not be prima facie obvious to combine the teachings of Kiessling with Amicucci as the DNA in the two methods is quite distinct (*i.e*, DNA in Amicucci *et al.* is free and circulating outside of a cell, while the DNA in Kiessling is released from or analyzed in a fixed cell).

One of ordinary skill in the art would not be motivated to combine the teachings of Amicucci *et al.* and Kiessling as the DNA in Amicucci has already been released from cells. Kiessling teaches adding agents to fix leukocytes (or other cells). Prior to the Applicants' invention, this methodology would be meaningless to one of ordinary skill in the art working with free nucleic acid (*i.e.*, nucleic acid already released from the cell).

Furthermore, even assuming one of ordinary skill in the art did want to reduce the risk of exposure to infectious agents when performing the assay disclosed in Amicucci *et al.*, there are numerous methods available to achieve this goal, and there is no motivation to choose the method disclosed by Kiessling. For example, U.S. Patent No. 5,985,260 discloses a method of disinfecting blood and blood components comprising preparing and immediately adding active albumin-iodine complex to the material to be disinfected. In addition, Moreton and Delves report that the addition of Virkon, which is a viral disinfectant, to clinical samples can reduce the likelihood of infection (J. Anal. At. Spectrom, 1999, 14:893-894). Thus, even if one of ordinary skill in the art was concerned with reducing the risk of exposure to infections agents, there are numerous methods available and there is nothing that would lead one of ordinary skill in the art to the disclosure of Kiessling.

The methods disclosed in claims 58-65, 67-68, 87-102, 190-194, 196 and 201 serve a long-felt need in the medical community, and provide unexpected results, and are therefore non-obvious. In addition, there is no motivation to combine the disclosures of Amicucci *et al.* and Kiessling, thus, Applicant respectfully requests that the rejection of claims 58-65, 67-68, 87-102, 190-194, 196 and 201 under 35 U.S.C. § 103 be withdrawn.

*Claims 66 and 195*: The Office rejected claims 66 and 195 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Kiessling [US Patent No. 5,618,664] as applied against Claim 60 above and further in view of Lo et al. [The Lancet 350: 485-487 (1997)]. Applicant respectfully traverses this rejection.

Claims 66 and 195 depend indirectly from amended claim 58 discussed above. Claim 66 and 195 further recite that the template DNA is obtained from serum.

As discussed above with respect to the rejection of claims 58-65, 67-68, 87-102, 190-194, 196 and 201 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Kiessling, the methods recited in claims 66 and 95 produce unexpected results and serve a long-felt need in the medical community. Applicant has discovered that the addition of a cell lysis inhibitor to a sample prior to determining the sequence of a locus of interest on free fetal DNA can significantly and unexpectedly increase the proportion of fetal DNA versus maternal DNA obtained from a sample. This result was unexpected, and therefore non-obvious (*see* M.P.E.P. 716.02(a)). Furthermore, Applicant respectfully submits that there is no motivation to combine the teachings of the reference Amicucci *et al.* with the teachings of the reference Kiessling as applied against claim 58 and further in view of Lo *et al.* because, *inter alia*, the methods disclosed in Amicucci *et al.* encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA.

Since the methods recited in claims 66 and 95 produce unexpected results and serve a long-felt need in the medical community, and since there is no motivation to combine Amicucci *et*

*al.* and Kiessling as applied against Claim 60 above and further in view of Lo et al., Applicant respectfully requests that the rejection of claims 66 and 195 under 35 U.S.C. § 103 be withdrawn.

*Claims 69-70 and 83*: The Office rejected claims 69-70 and 83 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Kiessling [US Patent No. 5,618,664] as applied against Claim 58 above and further in view of Saiki *et al.* [NEJM 319: 537-541 (1988)]. Applicant respectfully traverses this rejection.

Claims 69, 70 and 83 depend from amended claim 58 discussed above. Claim 69 further defines said locus of interest as a single nucleotide polymorphism. Claim 70 further defines said locus of interest as a mutation. Claim 83 recites specified methods for defining the locus of interest.

As discussed above with respect to the rejection of claims 58-65, 67-68, 87-102, 190-194, 196 and 201 under 35 U.S.C. § 103 as being unpatentable over Amicucci et al. in view of Kiessling, the methods recited in claims 69, 70 and 83 produce unexpected results and serve a long-felt need in the medical community. Applicant has discovered that the addition of a cell lysis inhibitor to a sample prior to determining the sequence of a locus of interest on free fetal DNA can significantly and unexpectedly increase the proportion of fetal DNA versus maternal DNA obtained from a sample. This result was unexpected, and therefore non-obvious (see M.P.E.P. 716.02(a)).

Furthermore, Applicant respectfully submits that there is no motivation to combine the teachings of the reference Amicucci et al. with the teachings of the reference Kiessling as applied against claim 58 and further in view of Saiki et al. because, inter alia, the methods disclosed in Amicucci et al. encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA. Saiki et al., alone or in combination with Amicucci et al. and Kiessling, does not teach or suggest the claimed methods

Since the methods recited in claims 69, 70 and 83 produce unexpected results and serve a long-felt need in the medical community, and since there is no motivation to combine Amicucci *et*

*al.* and Kiessling as applied against Claim 58 above and further in view of Saiki *et al.*, Applicant respectfully requests that the rejection of claims 69-70 and 83 under 35 U.S.C. § 103 be withdrawn.

*Claims 71-83*: The Office rejected claims 71-83 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Kiessling [US Patent No. 5,618,664 (1988)] as applied against Claim 58 above and further in view of Jones *et al.* [US 2003/0082576]. Applicant respectfully traverses this rejection.

Claims 71-83 all depend (directly or indirectly) from amended claim 58 discussed above. Claim 71 recites that the sequences of multiple loci of interest are determined. Claim 72 depends from claim 71 and recites that the loci of interest are on multiple chromosomes. Claims 73 and 74 recite methods for determining the sequence, and claims 75-83 recite additional elements for determining the sequence of the locus of interest.

As discussed above with respect to the rejection of claims 58-65, 67-68, 87-102, 190-194, 196 and 201 under 35 U.S.C. § 103 as being unpatentable over Amicucci et al. in view of Kiessling, the methods recited in claims 71-83 produce unexpected results and serve a long-felt need in the medical community. Applicant has discovered that the addition of a cell lysis inhibitor to a sample prior to determining the sequence of a locus of interest on free fetal DNA can significantly and unexpectedly increase the proportion of fetal DNA versus maternal DNA obtained from a sample. This result was unexpected, and therefore non-obvious (see M.P.E.P. 716.02(a)).

Furthermore, Applicant respectfully submits that there is no motivation to combine the teachings of the reference Amicucci et al. with the teachings of the reference Kiessling as applied against claim 58 and further in view of Jones et al. because, inter alia, the methods disclosed in Amicucci et al. encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA. Jones et al., alone or in combination with Amicucci et al. and Kiessling, does not teach or suggest the claimed methods.

Since the method recited in claims 71-83 produce unexpected results and serve a long-felt need in the medical community and since there is no motivation to combine Amicucci *et al.* and Kiessling as applied against Claim 58 above and further in view of Jones *et al.*, Applicant respectfully requests that the rejection of claims 71-83 under 35 U.S.C. § 103 be withdrawn.

*Claims 132 and 134-142*: The Office rejected claims 132 and 134-142 under 35 U.S.C. § 103as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] as applied against Claim 1 above and further in view of Chen *et al.* [Genome Research 10: 549-557 (2000)]. Applicant respectfully traverses this rejection.

Claims 132 and 134-142 depend (directly or indirectly) from amended claim 1. Claim 132 further defines the method used to determine the sequence of the locus of interest. Claims 134-142 recite further elements of the method used to determine the sequence of the locus of interest.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Amicucci *et. al*, alone or in combination with Saiki *et al.* as applied against Claim 1 and further in view of Chen *et. al* does not teach or suggest all elements of claims 132 and 134-142. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Chen *et al.*, either alone or in combination with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et al.* in view of Saiki *et al.* as applied against claim 1 and further in view of Chen *et. al* does not teach or suggest each and every element of claims 132 and 134-142, Applicant respectfully submits that the rejection of claims 132 and 134-142 under 35 U.S.C. § 103 be withdrawn.

*Claims 133-142 and 206*: The Office rejected claims 133-142 and 206 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in

view of Kiessling [US Patent No. 5,618,664] as applied against Claim 58 above and further in view of Chen *et al.* [Genome Research 10: 549-557 (2000)]. Applicant respectfully traverses this rejection.

Claims 133-142 and 206 depend (directly or indirectly) from amended claim 58 discussed above. Claim 133 defines a method for determining the sequence of the locus of interest. Claims 134-142 further recite elements of the method used to determine the sequence of the locus of interest. Claim 206 recites that the agent added to inhibit cell lysis is a cell lysis inhibitor.

As discussed above with respect to the rejection of claims 58-65, 67-68, 87-102, 190-194, 196 and 201 under 35 U.S.C. § 103 as being unpatentable over Amicucci et al. in view of Kiessling, the methods recited in claims 133-142 and 206 produce unexpected results and serve a long-felt need in the medical community. Applicant has discovered that the addition of a cell lysis inhibitor to a sample prior to determining the sequence of a locus of interest on free fetal DNA can significantly and unexpectedly increase the proportion of fetal DNA versus maternal DNA obtained from a sample. This was an unexpected result, and therefore non-obvious (see M.P.E.P. 716.02(a)).

Furthermore, Applicant respectfully submits that there is no motivation to combine the teachings of the reference Amicucci et al. with the teachings of the reference Kiessling as applied against claim 58 and further in view of Chen et al. because, inter alia, the methods disclosed in Amicucci et al. encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA.

Since the methods recited in claims 133-142 and 206 produce unexpected results and serve a long-felt need in the medical community, and since there is no motivation to combine Amicucci *et al.* and Kiessling as applied against claim 58 and further in view of Chen *et al.*, Applicant respectfully requests that the rejection of claims 133-142 and 206 under 35 U.S.C. § 103 be withdrawn.

*Claims 143 and 145-146*: The Office rejected claims 143 and 145-146 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] as applied against Claim 1 above and further in view of Livak *et al.* [U.S. Patent No. 5,538,848]. Applicant respectfully traverses this rejection.

Claims 143 and 145-146 depend (directly or indirectly) from amended claim 1 discussed above. Claim 143 defines a method used to determine the sequence of the locus of interest. Claims 145 and 146 recite further elements of the method used to determine the sequence of the locus of interest.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Amicucci *et. al*, alone or in combination with Saiki *et al.* as applied against Claim 1 and further in view of Livak *et. al* does not teach or suggest all elements of claims 143 and 145-146. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Livak *et al.*, either alone or in combination with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et al.* in view of Saiki *et al.* as applied against claim 1 and further in view of Livak *et. al* does not teach or suggest each and every element of claims 143 and 145-146, Applicant respectfully submits that the rejection of claims 143 and 145-146 under 35 U.S.C. § 103 be withdrawn.

*Claims 144-146 and 206*: The Office rejected claims 144-146 and 206 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Kiessling [US Patent No. 5,618,664] as applied against Claim 58 above and further in view of Chen *et al.* [Genome Research 10: 549-557 (2000)]. Applicant respectfully traverses this rejection.

Claims 144-146 and 206 all depend (directly or indirectly) from claim 58 discussed above. Claims 144-146 define a method used to determine the sequence of the locus of interest. Claim 206 recites a particular type of agent used to prevent lysis of cells.

As discussed above with respect to the rejection of claims 58-65, 67-68, 87-102, 190-194, 196 and 201 under 35 U.S.C. § 103 as being unpatentable over Amicucci et al. in view of Kiessling, the methods recited in claims 144-146 and 206 produce unexpected results and serve a long-felt need in the medical community. Applicant has discovered that the addition of a cell lysis inhibitor to a sample prior to determining the sequence of a locus of interest on free fetal DNA can significantly and unexpectedly increase the proportion of fetal DNA versus maternal DNA obtained from a sample. This was an unexpected result, and therefore non-obvious (see M.P.E.P. 716.02(a)).

Furthermore, Applicant respectfully submits that there is no motivation to combine the teachings of the reference Amicucci et al. with the teachings of the reference Kiessling as applied against claim 58 and further in view of Chen et al. because, inter alia, the methods disclosed in Amicucci et al. encompass free DNA while the method disclosed in Kiessling encompass fixing cells and then lysing cells to analyze the analytes therein, which would be of no utility to one of ordinary skill working with free DNA. Chen et al., alone or in combination with Amicucci et al. and Kiessling, does not teach or suggest the claimed methods.

Since the methods recited in claims 144-146 and 206 produce unexpected results and serve a long-felt need in the medical community, and since there is no motivation to combine Amicucci *et al.* and Kiessling as applied against claim 58 and further in view of Chen *et al.*, Applicant respectfully requests that the rejection of claims 144-146 and 206 under 35 U.S.C. § 103 be withdrawn.

*Claims 148-151*: The Office rejected claims 148-151 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] and Chen *et al.* [Genome Research 10: 549-557 (2000)] as applied

against Claim 132 above and further in view of Kiessling [US Patent No. 5,618,664]. Applicant respectfully traverses this rejection.

Claims 148-151 all depend (indirectly) from amended claim 1 discussed above. Claims 148-151 further recite that an agent that inhibits cell lysis is added to the sample.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Amicucci *et. al*, alone or in combination with Saiki *et al.* and Chen *et al.* as applied against claim 132 and further in view of Kiessling does not teach or suggest all elements of claims 148-151. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Chen *et al.* or Kiessling., either alone or in combination with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et al.* in view of Saiki *et al.* and Chen *et al.* as applied against claim 132 and further in view of Kiessling does not teach or suggest each and every element of claims 148-151, Applicant respectfully submits that the rejection of claims 148-151 under 35 U.S.C. § 103 be withdrawn.

*Claims 148-151*: The Office rejected claims 148-151 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* [Clinical Chemistry 46(2): 301-302 (2000)] in view of Saiki *et al.* [NEJM 319: 537-541 (1988)] and Livak *et al.* [U.S. Patent No. 5,538,848] as applied against claim 143 and further in view of Kiessling [US Patent No. 5,618,664]. Applicant respectfully traverses this rejection.

Claims 148-151 all depend (indirectly) from amended claim 1 discussed above. Claims 148-151 further recite that an agent that inhibits cell lysis is added to the sample.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Amicucci *et. al*, alone or in combination with Saiki *et al.* and Livak *et al.* as applied against claim 143 and further in view of Kiessling does not teach or suggest all elements of claims 148-151. As discussed above, with respect to the rejection of claims 1-4, 8, 52, 56-57, 152, 181-183, 185-187, 202-203, 205 and 207-208 under 35 U.S.C. § 103 as being unpatentable over Amicucci *et al.* in view of Saiki *et al.*, neither Amicucci *et al.* nor Saiki *et al.* teach or suggest quantitating a ratio of the relative amount of alleles in a mixture of maternal DNA and fetal DNA. Furthermore, this element is neither taught nor suggested by Livak *et al.* or Kiessling, either alone or in combination with Amicucci *et al.* or Saiki *et al.*

Since Amicucci *et al.* in view of Saiki *et al.* and Livak *et al.* as applied against claim 143 and further in view of Kiessling does not teach or suggest each and every element of claims 148-151, Applicant respectfully submits that the rejection of claims 148-151 under 35 U.S.C. § 103 be withdrawn.

*Claims 87-96*: The Office rejected claims 87-96 under 35 U.S.C. § 103 as being unpatentable over Kiessling [US Patent No. 5,618,664]. Applicant respectfully traverses this rejection.

Amended claim 87 is described above. Claims 88-94 depend from claim 87 and recite sources from which the sample is obtained. Claims 95 and 96 depend from claim 87 and recite cell lysis inhibitors.

As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Kiessling as applied against claims 87-96 does not teach or suggest all elements of claims 87-96. Amended claim 87 encompasses a method for preparing a sample for analysis comprising isolating free fetal nucleic acid from said sample, wherein said sample comprises an agent that inhibits cell lysis. Conversely, Kiessling discloses, for instance, a method for simultaneously disinfecting a biological sample,

lysing the red blood cells and fixing the leukocytes (col. 3, lines 26-29). Nowhere does Kiessling teach or suggest a method for preparing a sample for analysis comprising isolating free nucleic acid.

Kiessling discloses a method that comprises fixing cells, lysing the cells to release analytes, and then detecting the analytes. For instance, Kiessling states "Protease digested, ***formalin-fixed cells*** were initially subjected to PCR.... (col. 10, lines 50-51, emphasis added). Similarly, at col. 13, lines 63-65, Kiessling states "PCR amplification of HIV DNA sequences in ***formaldehyde fixed peripheral blood cells***...." Again, at col. 13, line 67 through col. 14, line 2, Kiessling states "DNA amplified sequences from ***formaldehyde fixed white blood cells*** of an HIV seropositive...." Kiessling analyzes nucleic acid from fixed cells; this nucleic acid is not free nucleic acid but rather nucleic acid within cells or nucleic acid that is then released. Kiessling fails to teach or suggest a method that isolates free nucleic acid.

Since Kiessling does not teach or suggest each and every element of claims 87-96, Applicant respectfully submits that the rejection of claims 87-96 under 35 U.S.C. § 103 be withdrawn.

*Claim 94:* The Office rejected claim 94 under 35 U.S.C. § 103 as being unpatentable over Kiessling [US Patent No. 5,618,664] as applied against Claim 93 above and further in view of Lo et al. [U.S. Patent No. 6,156,504 (2000)].

Claim 94 depends from claim 87, and as such incorporates all elements of claim 87 and further recites that the source of the sample is from plasma obtained from blood. As already noted, to establish a prima facie case of obviousness, the cited art must teach or suggest each and every claim limitation. Applicant respectfully submits that Kiessling as applied against claims 93 and further in view of Lo et al. does not teach or suggest all elements of claim 94. As discussed above with regard to the rejection of claims 87-96 under 35 U.S.C. § 103 as being unpatentable over Kiesssling, Kiessling fails to teach or suggest a method for preparing a sample for analysis comprising isolating free fetal nucleic acid from said sample, wherein an agent that inhibits cell lysis has been added to the sample. Rather, Kiessling discloses, e.g., a method for simultaneously

disinfecting a biological sample, lysing the red blood cells and fixing the leukocytes (col. 3, lines 26-29). Nowhere does Kiessling teach or suggest a method for preparing a sample for analysis comprising isolating free nucleic acid.

Since Kiessling as applied against claim 93 and further in view of Lo *et al.* does not teach or suggest each and every element of claim 94, Applicant respectfully submits that the rejection of claim 94 under 35 U.S.C. § 103 be withdrawn.

## CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 543312000420. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: May 30, 2007

Respectfully submitted,

By

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